COMPOSITIONS AND METHODS FOR ENZYMATIC DISRUPTION OF BACTERIAL BIOFILMS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a national stage entry under U.S.C. § 371 of International Application No. PCT/US2019/054868, filed Oct. 4, 2019, which in turn claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Ser. No. 62/742,158, filed Oct. 5, 2018, the contents of each of which are hereby incorporated by reference in its entirety into the present disclosure.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under Grant No. R01DC011818 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Apr. 1, 2021, is named 106887-7861_SL.txt and is 19,024 bytes in size.

BACKGROUND

[0004] Bacteria adopt a biofilm state that represents multicellular microbial communities adherent to each other as well as to an abiotic or biotic surface. Bacteria in a biofilm are surrounded by extracellular polymeric substances, primarily comprised of exopolysaccharides, extracellular DNA (eDNA) and proteins. eDNA is ubiquitous and a pivotal component to maintain the structural integrity of bacterial biofilms.

[0005] Bacteria persisting in a biofilm in the human body cause about two-thirds of all chronic/recurrent diseases. These biofilms are comprised of bacteria protected by an outer "slime" that is often comprised primarily of DNA that prevents the innate and adaptive immune systems, antibiotics and other antibacterial agents from gaining access to the bacteria inside the biofilm, making it extremely difficult to clear the infection from the body. Furthermore, the biofilm can act as a reservoir for future acute infections often with lethal consequences. Biofilms are present in an industrial setting as well. For example, biofilms are implicated in a wide range of petroleum process problems, from the production field to the gas station storage tank. In the field, sulfate reducing biofilm bacteria produce hydrogen sulfide (soured oil). In the process pipelines, biofilm activity develops slimes which impede filters and orifices. Biofilm and biofilm organisms also cause corrosion of pipeline and petroleum process equipment. These problems can be manifested throughout an oil or gas production facility to the point where fouling and corrosive biofilm organisms have even been found on the surfaces of final product storage tanks.

[0006] In the home, biofilms are found in or on any surface that supports microbial growth, e.g., in drains, on food preparation surfaces, in toilets, and in swimming pools and spas.

[0007] Biofilms are implicated in a wide range of water processes, both domestic and industrial. They can grow on the surface of process equipment and impede the performance of the equipment, such as degradation of heat transfer or plugging of filters and membranes. Biofilms growing on a cooling tower fill can add enough weight to cause collapse of the fill. Biofilms cause corrosion of even highly specialized stainless steels. Biofilms in a water process can degrade the value of a final product such as biofilm contamination in a paper process or the attachment of even a single cell on a silicon chip. Biofilms growing in drinking water distribution systems can harbor potential pathogenic organisms, corrosive organisms or bacteria that degrade the aesthetic quality of the water.

SUMMARY

[0008] Bacteria adopt a biofilm state that represents multicellular microbial communities adherent to each other as well as to an abiotic or biotic surface. Bacteria in a biofilm are surrounded by extracellular polymeric substances, primarily comprised of exopolysaccharides, extracellular DNA (eDNA) and proteins. eDNA is ubiquitous and a pivotal component to maintain the structural integrity of bacterial biofilms. Applicant have shown previously that eDNA in biofilms formed by multiple bacterial species is organized into a lattice-like structure that is stabilized by DNABII proteins. DNABII proteins are a family of DNA binding proteins that exhibit high affinity to pre-bent DNA.

[0009] Thus, in one aspect, provided herein is a method to inhibit or disrupt a biofilm, the method comprising, or alternatively consisting essentially of, or yet further consisting of, contacting the biofilm with an agent that cleaves the Holliday junction (HJ) structure in the biofilm. Non-limiting examples of such agents include RusA polypeptide or a RuvABC peptide complex. In one aspect, the contacting is in vitro in a test tube or ex vivo. In another aspect, the contacting is in vivo, and the contacting is achieved by administering the agent to a subject in need thereof. The biofilm or diseases incident to a biofilm infection that can be treated by these methods can be caused by bacterial infections, e.g., infections by the ESKAPE pathogens, UPEC, NTHI, *S. epidermidis, Streptococcus agalactiae, Neisseria meningitidis*, Treponemes, *denticola, pallidum*), *Burkholderia cepacia*), or *Burkholderia pseudomallei, Haemophilus influenzae* (nontypeable), *Moraxella catarrhalis, Streptococcus pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Mycobacterium tuberculosis,*

[0010] In one aspect, the agent for use in the method comprises, or consists essentially of, or yet consists of an HJ-specific endonuclease, for example RuvABC or RusA.

[0011] Also provided is a method to inhibit or disrupt a biofilm or treat a disease or condition incident to a biofilm infection in a subject in need thereof, the method comprising, or alternatively consisting essentially of, or yet further consisting of, administrating to the subject an effective amount of an agent that cleaves the Holliday junction (HJ) structure in the biofilm. The agent can be administered locally or systemically. In one aspect, the agent for use in the method comprises, or consists essentially of, or yet consists of an HJ-specific endonuclease, for example RuvABC or RusA.

[0012] In one aspect, the method is used to treat mammals, human patients, or pediatric mammals or human patients.